

Rapid staining technique for *Trichomonas vaginalis*

Preliminary report

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SUMMARY A simple, rapid, staining method for the identification of *Trichomonas vaginalis* has been tested on cultured trichomonads and specimens of vaginal discharge. Fifty-eight stained slides of vaginal discharge were examined and trichomonads were correctly identified in the 31 specimens from patients with confirmed vaginal trichomoniasis. No false-positive results were obtained. This staining procedure could prove a useful addition to wet-film and cultural methods.

Introduction

Trichomoniasis is a common cause of genitourinary symptoms in women and may be responsible for some cases of nongonococcal urethritis in men. Usually diagnosis in women is easily made by examination of a specimen of vaginal discharge diluted in isotonic saline on a slide using phase-contrast or darkground microscopy. A carefully prepared and examined wet film is considered to give as accurate results as cultural methods (Morton, 1976). In a few cases in women (Gardner and Kaufman, 1969a) and in most cases in men (Candiani *et al.*, 1973) the trichomonads are not easily recognisable because they are few, non-motile, and in an encysted form.

Staining methods for the recognition of *Trichomonas vaginalis*, such as Papanicolaou or Giemsa, are not of use in routine clinical settings because they are too time-consuming (Gardner and Kaufman, 1969b). The recognition of *T. vaginalis* in Gram-stained smears was described by Cree (1968), but this method was not considered of use by Thin and Michael (1970).

This preliminary report describes a simple, rapid, staining method for trichomonads which can be used routinely in a department of genitourinary medicine.

Materials and methods

The staining method used is based on the Diff-

Quik*, a rapid differential stain set developed for use in haematology.

To develop the stain for detecting *T. vaginalis* it was initially used on cultured trichomonads until optimal staining results were obtained. Specimens of vaginal discharge from patients were then examined. A platinum-loop specimen of discharge (or Feinberg-Whittington culture medium containing trichomonads) was mixed on a degreased slide with a drop of saline and spread thinly. It was then air-dried in warm conditions and stained.

STAINING TECHNIQUE

The slide was dipped consecutively in the following reagents of the Diff-Quik set:

- (1) Arylmethane dye, 0.002 g/l in methanol (eight dips of one second each);
- (2) Xanthene dye, 1.25 g/l in phosphate buffer, pH 6.60 (five dips of one second each);
- (3) Thiazine dye, 1.25 g/l in phosphate buffer, pH 6.60 (five dips of one second).

The slide was then washed in phosphate buffer, pH 7.0, and blotted dry. The whole procedure took two to five minutes, depending on the drying process and the thickness of the smear. The slide was then examined microscopically using an oil immersion objective ($\times 500$ or $\times 1000$ magnification).

PATIENTS

Fifty-one women were studied, aged 17 to 38 years; they were of English origin except for three from the West Indies. Twenty-seven women were selected because vaginal trichomoniasis was suspected on

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Table Results of staining of specimens of vaginal discharge

Diagnosis	Diff-Quik stain			Wet film (positive results)			
	+	—	Total	Alone	With culture	Culture alone	Total
Trichomoniasis	31	0	31	4	26	1	31
Other	0	27	27				
Total	31	27	58				

+ positive —negative

clinical grounds, and in 31 specimens from 24 of these women trichomonads were identified on wet film or culture or both. Twenty-seven specimens were obtained from a further 24 women selected as controls. These women did not have trichomoniasis but attended either as contacts of men with non-specific urethritis, or because they had candidosis or genital warts, or for examination to exclude disease. Thus, 58 specimens of vaginal discharge were examined from the 51 women studied.

Results

After preliminary trials to obtain optimal results, 30 smears of cultured trichomonads were stained by the

technique described. Similar stains were carried out on the 58 specimens of vaginal discharge. These were examined microscopically by a technician who had no previous knowledge of the diagnosis. The results are shown in the Table. All the known cases of vaginal trichomoniasis were identified by the Diff-Quik staining method. Up to two minutes' microscopy was allotted to each stained slide, but stained trichomonads were recognised in most known cases in the first few fields. We were unable to identify the trichomonads in Gram stains of the same material with any consistency.

The trichomonad was readily identified by its light-blue cytoplasm, red flagellae and axostyle, and purple oval nucleus in the forward part of the



Fig. 1 Four trichomonads with epithelial cells and a polymorph (upper right centre). The four flagellae and undulating membrane of one trichomonad are clearly shown (Diff-Quik stain of vaginal discharge).



Fig. 2 Four trichomonads with six polymorphs and cellular debris. The lower-right trichomonad clearly shows the nucleus, cytoplasm, axostyle, flagellae, and undulating membrane (Diff-Quik stain of vaginal discharge).

protozoon (Figs. 1 and 2). The undulating membrane was usually also seen and stained red. Even in encysted forms the flagellae and nucleus were readily identified, thus enabling differentiation from epithelial and white blood cells.

Cultured trichomonads were also easily recognised after being added to specimens of urethral discharge taken from six men with non-specific urethritis.

Discussion

This preliminary report shows that trichomonads can be easily identified by this staining method. All known cases of trichomoniasis were positively identified and one case (female) was diagnosed even when wet-film methods and initial culture gave negative results. The results are comparable with those of the Giemsa method but are more rapidly obtained.

The stain should prove useful in women with features suggesting trichomoniasis but in whom no trichomonads are identified on the wet film. (The wet film itself can be stained, thus obviating the need for further samples). Other patients who would be suitable for investigation by this method could include women with inflammatory cervical smears of

unknown origin and men with non-specific urethritis—particularly those cases which are recurrent and unresponsive to routine therapy. Smears of urethral and prostatic material or urinary sediment could be used in the latter cases. The method could prove useful as an adjunct to cultures for trichomoniasis, particularly for patients seen in general practice, where smears as well as cultures could be sent to the laboratory.

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